SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 21, No. 9, pp. 1783–1787, 2002
Printed in the USA
0730-7268/02 $9.00 + .00

COMPARISON OF POLYCYCLIC AROMATIC HYDROCARBON LEVELS IN MUSSELS AND OYSTERS IN FRANCE AND THE UNITED STATES

BENOIT BELIAEFF,*† THOMAS P. O'CONNOR,‡ CATHERINE MUNSCHY,† BERNARD RAFFIN,† and DIDIER CLAISSE†
†IFREMER, B.P. 21105, F-44311 Nantes Cedex 3, France
‡National Oceanic and Atmospheric Administration N/NSC1, 1305 East West Highway, Silver Spring, Maryland 20910, USA

(*Received* 13 *December* 2001; *Accepted* 1 *March* 2002)

Abstract—This paper compares data of 15 individual polycyclic aromatic hydrocarbon (PAH) concentrations from two monitoring programs: the French Réseau National d'Observation de la qualité du milieu marin (RNO) and the Mussel Watch project of the U.S. National Status and Trends (NST) program. Polycyclic aromatic hydrocarbon measurements in bivalve (mussels and oysters) are made from samples collected at 265 sites along the U.S. coastline and at 97 sites in the French coastal waters. Individual PAH patterns were found strikingly similar between the two countries with higher concentrations for high-molecular-weight (HMW) PAHs. Principal component analysis results for both RNO and NST show the variability to be dominated by just two components with HMW compounds contributing primarily to the first and low-molecular-weight (LMW) compounds to the second. This could imply a separation of petrogenic and pyrolytic sources with the latter being the more important in both nations accounting for the similarity in results.

Keywords—Polycyclic aromatic hydrocarbons Monitoring Mussel watch Bivalves Coastal waters

INTRODUCTION

Since 1979, the Réseau National d'Observation de la qualité du milieu marin (RNO) has been collecting and analyzing mussels and oysters from sites along the French coast. In the United States, the National Oceanic and Atmospheric Administration NST Mussel Watch Project has been doing the same thing since 1986. Both programs monitor spatial and temporal trends of contamination in coastal areas using mussels and oysters as sentinel organisms [1,2]. Beliaeff et al. [3] reported on the national scale similarities and differences in concentrations of Cd, Hg, Pb, Cu, Zn, lindane (gamma-hexachlorocyclohexane), and ΣDDT (dichloro-diphenyl-trichlorethane and its degradation products) measured from 1986 to 1993 by these two programs. Here we compare results on concentrations of 15 PAHs included in the RNO project since 1994 (Table 1). They have been part of the NST suite of chemicals since 1986, and O'Connor [4] concluded that they represent the one chemical group among all the NST analytes that might be eliciting a biological response at the concentrations found in some mussels and oysters. Polycyclic aromatic hydrocarbons are also the major contributor to the total induction equivalent factor (ΣIEF) in mussels and oysters [5] because they are found at high concentrations relative to other CYP1A inducers such as dioxins and planar PCBs that are more potent on a molar basis.

Polycyclic aromatic hydrocarbons are of special interest in monitoring programs because of their persistence and bioaccumulative properties, especially in marine mollusks, which have limited ability to metabolize these compounds as compared to fish [6]. Major sources of PAHs in the environment are the production, transport, and use of fossil fuels (petrogenic PAHs) and the incineration of organic materials, for example, fossil fuels, wood, and waste (pyrogenic PAHs). Petrogenic PAHs are predominated by LMW with two or three aromatic rings, while pyrogenic PAHs typically contain higher portions of higher-molecular-weight compounds with four and five rings (Table 1). Some high-molecular-weight (HMW) PAHs, such as benzo[*a*]pyrene, are recognized to be mutagenic and/or potentially carcinogenic [7] and thus are classified as environmental priority pollutants by the U.S. Environmental Protection Agency [8] and the European Community [9].

Through the comparison of levels and distributions for a prioritary pollutant, this paper aims at studying the coherence of results issued from two monitoring programs structured by the same objectives and, therefore, by the same type of sampling strategies. Specifically, the origin of PAHs in coastal waters will be investigated.

MATERIALS AND METHODS

Data

The comparison is done with data from samples collected in the winters of 1994 to 1995, 1995 to 1996, 1996 to 1997, and 1997 to 1998. The RNO data are from 97 sites sampled in November or December. The NST data are from 265 sites sampled in November to March (each site sampled within 30 d of a prescribed date). The NST data are available at http://www.ccma.nos.noaa.gov and the RNO data at http://www.ifremer.fr/envlit. The RNO protocol requires that each site be sampled every year. Since 1993, the NST practice has been to sample sites every other year. Over four years, data are available for more than one year for all the RNO and NST sites. The comparisons begin then by calculating mean concentrations for PAHs at each site in each nation.

Site distributions and species

The NST sites are located about 70 km apart along the U.S. coast and 20 km apart within estuaries (Fig. 1). A bias exists toward urban areas in the NST station grid since 45% of the sites are located within 20 km of population centers with 100,000 and more inhabitants. The RNO sites are more evenly

* To whom correspondence may be addressed (benoit.beliaeff@ifremer.fr).

Table 1. List of polycyclic aromatic hydrocarbons (PAH) common to the Réseau National d'Observation (RNO) and to the National Status and Trends (NST) project and corresponding codes. Polycyclic aromatic hydrocarbons are ranked and grouped according to their molecular weight (LMW = low molecular weight; HMW = high molecular weight)

PAH	Code	No. of rings	Molecular weight
LMW			
Naphthalene	NAPH	2	128
Acenaphthene	ACEE	3	154
Fluorene	FLUE	3	166
Anthracene	ANTH	3	178
Phenanthrene	PHEN	3	178
HMW			
Fluoranthene	FLUA	4	202
Pyrene	PYRE	4	202
Benzo[*a*]anthracene	BANT	4	228
Chrysene	CHRY	4	228
Benzo[*a*]pyrene	BAPY	5	252
Benzo[*b*]fluoranthene	BBFL	5	252
Benzo[*k*]fluoranthene	BKFL	5	252
Benzo[*ghi*]perylene	BPER	5	276
Indeno[1,2,3-*cd*]pyrene	INDE	6	276
Dibenzo[*a*,*h*]anthracene	DBAN	5	278

distributed, without any urban emphasis. In both monitoring programs, sites were selected to be representative of their surroundings, and point sources of chemical contamination were deliberately avoided.

The mussel watch programs are ongoing in both nations. Only indigenous organisms were collected. Among the 97 RNO sites, the mussels *Mytilus edulis* or *Mytilus galloprovincialis* were collected at the 61 sites along the Mediterranean and Northwest coasts and the oyster *Crassostrea gigas* at the 36 remaining Atlantic sites. Among the 265 NST sites, the 113 from Delaware Bay to Laguna Madre, Texas, USA, were sampled for the oyster *Crassostrea virginica*, the mussel *M. edulis* was collected at the 50 sites in the northeastern United States, and at the 69 West Coast sites both *M. edulis* and *Mytilus californianus* were sampled. Additionally, the oyster *Ostrea sandvicensis* was sampled at the three NST sites in Hawaii, USA; the smooth-edged jewel box *Chama sinuosa* at the one site in the Florida Keys, USA; and the mangrove oyster *Crassostrea rhizophorae* in three sites in Puerto Rico, USA. We have also included here data from the 25 NST sites on the freshwater Great Lakes where zebra mussels *Dreissena polymorpha* was sampled in summer (August and September). Chemical concentrations in mollusks from two sets of sites that allowed sampling of *M. edulis* and *M. virginica* or *M. edulis* and *M. californianus*, respectively, suggested species-independent bioaccumulation of organic contaminants including PAHs [10]. Therefore, mussel data were analyzed without regard to the respective species.

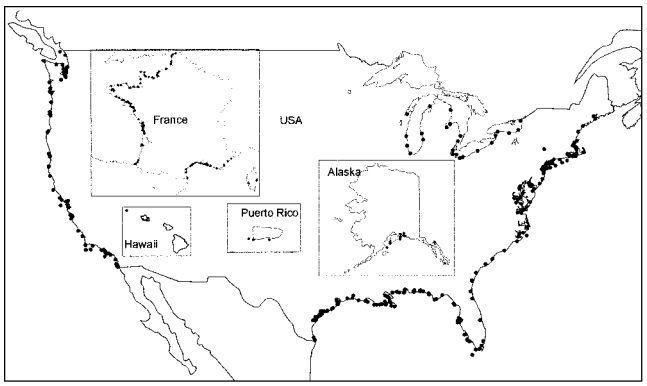

Fig. 1. Site location map for the National Status and Trends (NST) project and for the Réseau National d'Observation (RNO).

Analytical methods

All PAH analyses for NST between the years 1994 and 1998 were done by the Texas A&M University Geochemical and Environmental Research Group (GERG, College Station, TX, USA). Details are provided by Lauenstein and Cantillo [11]. Composites of whole soft parts of 20 oysters, 30 mussels, or about 100 zebra mussels were homogenized. After addition of internal standards and anhydrous sodium sulfate, the homogenate was extracted three times with dichloromethane using a tissumizer. After concentration by solvent evaporation and exchange of dichloromethane with hexane, the tissue extract was fractionated by alumina:silica chromatography. The aromatic fraction eluted from the column with 1:1 pentane: dichloromethane was further purified by removing lipids through the gel-permeation/high-performance liquid chromatography procedure developed by Krahn et al. [12]. Purified extracts were chromatographed on 30-m DB-5 fused silica capillary columns (J&W Scientific, Rancho Cordova, CA, USA) followed by mass spectroscopy detection (GC-MSD) used in the selected ion mode (SIM).

All analyses were performed by the Laboratoire Municipal et Régional of Rouen (France) for the RNO. Samples were hand collected once a year in November from more than 100 stations along the French coasts and left for 24 h in clean marine waters to purify. At least 10 oysters or 50 mussels were pooled as a composite sample at each site. Soft tissues were homogenized using a tissumizer, freeze-dried, and stored in a closed vessel until further analyses. Five grams of freeze-dried samples were extracted by hexane/acetone (50/50) using an accelerated solvent extractor (ASE 200, Dionex, Sunnyvale, CA, USA), concentrated in hexane, and fractionated by silica column chromatography. For individual PAH analyses, 10 μl of samples in acetonitrile were injected automatically and separated by reverse-phase high-performance liquid chromatography using a 25-cm × 4.6-mm column (Vydac 201 TP—C18, Separations Group, Hesperia, CA, USA) kept at 30°C and coupled with programmable wavelength fluorescence detection.

Comparability of analytical data provided by different laboratories was ensured by intercalibration for NST [13–16] and for RNO [17,18].

Statistical analysis

Polycyclic aromatic hydrocarbon distribution patterns can be visualized using box-and-whisker plots. The boxes cover interquartile range (the first to third quartiles, identically the 25th and 75th percentiles), with the horizontal line within each box indicating the median concentration. Vertical lines, so-called whiskers, extend from each end of the box to the lowest and highest observation within intervals defined as 1.5 interquartile ranges below the first and above the third quartile, respectively. Individual observations that are beyond these intervals are plotted as dots.

Principal component analysis (PCA) has been applied to tables consisting of 15 individual PAH compound concentrations at each of 97 (RNO) or 265 (NST) sites. Principal com-

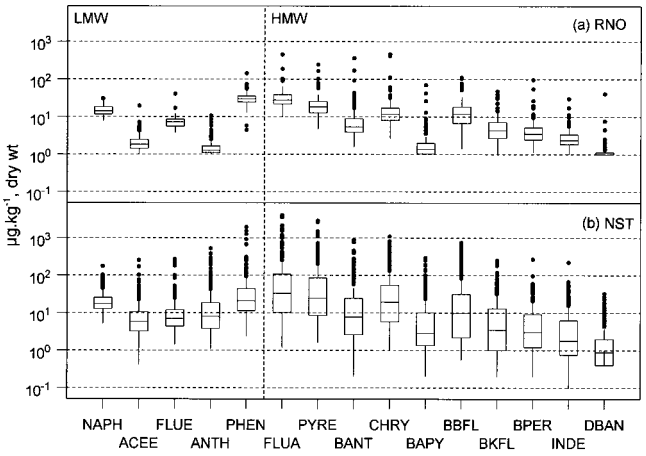

Fig. 2. Box-and-whisker plots of polycyclic aromatic hydrocarbon (PAH) concentration, expressed in µg/kg (dry wt) for the Réseau National d'Observation (RNO) (**a**) and for the National Status and Trends (NST) project (**b**) (NAPH = naphtalene, ACEE = acenaphthene, FLUE = fluorene, ANTH = anthracene, PHEN = phenanthrene, FLUA = fluoranthene, PYRE = pyrene, BANT = benzo[*a*]anthracene, CHRY = chrysene, BAPY = benzo[*a*]pyrene, BBFL = benzo[*b*]fluoranthene, BKFL = benzo[*k*]fluoranthene, BPER = benzo[*ghi*]perylene, INDE = indeno[1,2,3-*cd*]pyrene, DBAN = dibenzo[*a,h*]anthracene).

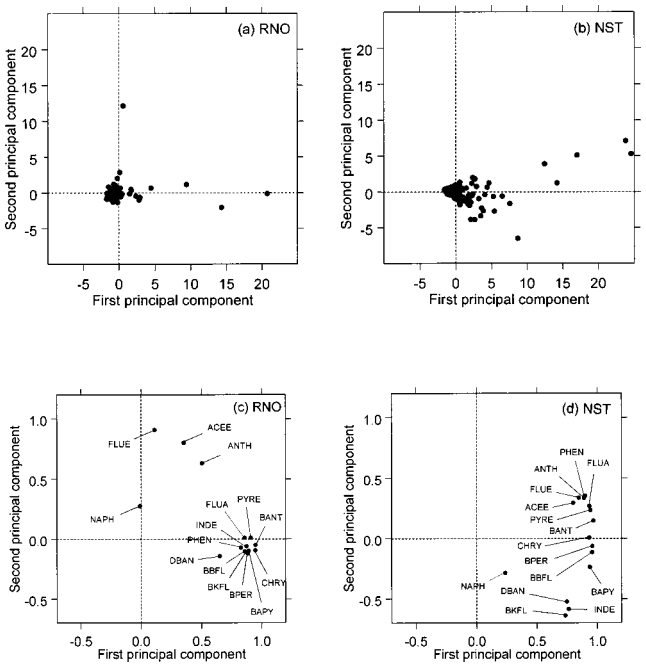

Fig. 3. Principal component analysis (PCA) results for the Réseau National d'Observation (RNO) (**a,c**) and for the National Status and Trends (NST) project (**b,d**). Top plots represent sampling site scores in the main factorial plane for RNO (**a**) and for the NST Project (**b**). Most sites are grouped near the origin showing similar patterns of individual polycyclic aromatic hydrocarbons (PAH). Outlying dots correspond to highly contaminated sites. Bottom plots correspond to individual PAH loadings (dot coordinates are the linear correlations of each individual PAH with the first two components) for RNO (**c**) and NST (**d**) (NAPH = naphtalene, ACEE = acenaphthene, FLUE = fluorene, ANTH = anthracene, PHEN = phenanthrene, FLUA = fluoranthene, PYRE = pyrene, BANT = benzo[*a*]anthracene, CHRY = chrysene, BAPY = benzo[*a*]pyrene, BBFL = benzo[*b*]fluoranthene, BKFL = benzo[*k*]fluoranthene, BPER = benzo[*ghi*]perylene, INDE = indeno[1,2,3-*cd*]pyrene, DBAN = dibenzo[*a,h*]anthracene). Lowest-molecular-weight PAHs appear separated from high-molecular-weight PAHs, showing lower correlation values with the first principal component.

ponent analysis axes are orthogonal and derived from a linear combination of the original variables (for thorough details on PCA see, e.g., Rao [19]). Classically, in order not to over- or underweigh any PAH on the basis of its absolute concentration range, concentrations have been standardized: For each individual PAH, the mean concentration is subtracted from every single value, and the result is divided by the corresponding standard deviation.

RESULTS

The box-and-whisker plots (Fig. 2a and b) show, on a logarithmic scale, the distribution of concentrations among the 15 PAHs for RNO and NST. The relative concentration distributions among the 15 compounds and the median concentrations for each compound are very similar (Fig. 2a and b). This is also the case when comparing medians of aggregate groups: sums of LMW (55 µg/kg, dry wt, for RNO, and 63 µg/kg, dry wt, for NST), sums of HMW (88 µg/kg, dry wt, for RNO and 124 µg/kg, dry wt, for NST), the total sum (138 µg/kg, dry wt, for RNO and 188 µg/kg, dry wt, for NST). Finally, ratios of LMW to HMW are identical between the two programs with a value of 0.58. The main difference between NST and RNO is for the high concentrations. The NST third quartiles are much higher than those of RNO, and NST includes many sites with PAH concentrations above the upper whisker.

For both countries, distributions are highly right skewed. The logarithmic scales for box plots force the distributions to appear symmetric, but skewness in our results remains evident as outlying observations above the upper part of the boxes for both countries (Fig. 2). This is also shown by much higher values for means than for medians. Frequency histograms for benzo[*a*]pyrene and fluoranthene demonstrated highly right-skewed distributions for both programs (figure not shown).

While box plots aggregate data for each individual polycyclic aromatic hydrocarbon, PCA takes the concentration of each individual PAH and each sample into account. Scores of sampling sites are represented in Figure 3a for RNO and in Figure 3b for NS&T. For both countries, the main factorial plane extracts a large part of the variability: 72% for RNO and 85% for NST, with, respectively, 58 and 74% for the first (horizontal) axis. Linear correlation of variables (four-letter codes of PAHs) with the first and the second principal components (loadings) are illustrated in Figures 3c and d for, respectively, RNO and NST. Small angles connecting (0, 0) with any two PAHs imply a similar contribution of those individual PAHs to the principal component axes. Variables contributing the most to axis 1 are HMW for both countries. For RNO and NST, the second (vertical) axis opposes HMW to LMW. This is especially striking for RNO, where naphtalene, fluorene, acenaphthene, and anthracene are clearly separated from the other PAHs. Log transformation of the data did not change the main features in the PCA results.

Close observations (dots) tend to follow the same pattern considering the available set of individual PAHs. Most of the sites are grouped near the origin exhibiting low between-site variability with similar patterns and concentrations. The outliers, showing high concentrations, also lie on the right part of the plane. All the NST sites that are separate from the central mass in Figure 3b are sites that O'Connor [4] characterized as high for HMW because mollusks consistently displayed

concentrations above the 85th percentile of the 1990 data for 24 PAHs. These U.S. sites also appear above the whiskers on Figure 2 and are sites near urban areas of Boston, Massachusetts; New York, New York; Panama City, Florida; Galveston Bay, Texas; San Diego, California; Los Angeles, California; San Francisco, California; Seattle, Washington; and on the Great Lakes near Milwaukee, Wisconsin, and Rochester, New York. No NST sites are distinguished by a high value only on axis 2 (LMW), but the French data for the site Centre Darse 2, located in the Gulf of Fos nearby petroleum refineries, lies high along axis 2, suggesting a strictly petrogenic influence. The dominance of HMWs is even more evident in the NST data for 24 PAHs that add seven LMW and two HMW compounds to the list of 15 used here. The PCA plot based on that larger data set (not shown) distinguishes two sites as particularly low in LMW compounds and still shows no sites dominated by the LMW compounds.

DISCUSSION AND CONCLUSIONS

On a qualitative basis, U.S. and French data exhibit almost identical PAH profiles (Fig. 2). The similarity is strikingly revealed by common sets of variables contributing to the first two axes of PCA (Fig. 3) with HMW compounds accounting for most of the variability. Because marine mollusks have only low PAH-metabolizing capacities, this distribution reflects the bioavailable part of the PAHs to which they have been exposed. The PAH distribution pattern is dominated by tetra-aromatic compounds in the molecular weight range of 202 to 228. These compounds account for 44 and 49% of ΣPAH for RNO and NST data, respectively. In addition, naphthalene concentrations show relatively high levels with around 10% (median values) of ΣPAH for both countries. Similar patterns observed in all samples from both programs suggest that mollusks are exposed to PAHs originating from similar sources. Predominance of HMW compared to LMW compounds, with identical LMW:HMW ratio medians for both monitoring programs (0.58), may indicate a predominant pyrolitic origin. Right skewness of LMW:HMW ratio distributions also expresses that most of PAH contamination is due to combustion of organic matter. Low molecular weight/high molecular weight is often used as an indicator of contamination sources [20], a low value indicating a pyrolitic influence. These results are consistent with the well-known ubiquity and predominance of combustion-derived PAHs in the marine environment [21], which results from both high inputs from various sources and slow degradation [22]. Additional information on alkyl homologues of PAH would certainly help in better discriminating pyrolytic and petrogenic sources.

For each country, sites are mostly grouped near the origin of the two-dimension PCA plots (Fig. 3a and c), further evidence of remarkable similarity. With one exception, sites distinctly away from the origin are those with exceptional high HMW concentrations in mollusks and are sites in urban and industrialized areas. As already indicated, the sampling strategy of both the RNO and the NST programs avoids direct sources of contamination. According to the mussel watch concept, site locations should not be hot spots, more likely to face chronic petrogenic contamination. On the contrary, these sites are subject to diffuse and complex mixtures issued mainly from combustion. The remarkable similarity in median concentrations implies that both programs are fairly sampling representative sites and that on national scales PAHs are dominated by HMW compounds in concentrations that may be typical of developed countries. The NST program has many more sites than RNO, and site selection is biased toward urban areas. The consequence is not that the medians are very different from RNO but that NST includes many more sites with concentrations at the high end of the overall distribution (Fig. 2). Moreover, broader concentration ranges for the NST data can also partly be explained because sampling in November to March (with respect to RNO sampling in November or December) may lead to higher seasonal variability due to significant differences in bivalve physiological conditions, such as filtration rate or spawning activity [23,24].

Acknowledgement—The authors are thankful to the 12 IFREMER Coastal Laboratories for sample collection. The authors are grateful to four anonymous reviewers for their relevant corrections and suggestions on the paper.

REFERENCES

1. Claisse D, Joanny M, Quintin JY. 1992. Le Réseau National d'Observation de la qualité du milieu marin (RNO). *Analusis* 20: 719–722.
2. O'Connor TP. 1996. Trends in chemical concentrations in mussels and oysters collected along the U.S. coast from 1986 to 1993. *Mar Environ Res* 41:183–200.
3. Beliaeff B, O'Connor TP, Claisse D. 1998. Comparison of chemical concentrations in mussels and oysters from the United States and France. *Environ Monit Assess* 49:87–95.
4. O'Connor TP. 2002. National distribution of chemical concentrations in mussels and oysters in the USA. *Mar Environ Res* 53: 117–143.
5. Gardinali PR, Wade TL. 1998. Contribution of PAHs, PCBs, and PCDD/PCDFs to the total induction equivalents (Sigma IEs) in mollusks. *Mar Pollut Bull* 37:27–31.
6. James MO. 1989. Biotransformation and disposition of PAH in aquatic invertebrates. In Varanasi U, ed, *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment.* CRC, Boca Raton, FL, USA, pp 69–92.
7. Marvin CH, Lundrigan JA, McCarry BE, Bryant DW. 1995. Determination and genotoxicity of high molecular mass polycyclic aromatic hydrocarbons isolated from coal-tar-contaminated sediment. *Environ Toxicol Chem* 14:2059–2066.
8. Keith LH, Teilliard WA. 1979. Priority pollutants I—A perspective view. *Environ Sci Technol* 13:416–423.
9. European Parliament and European Council. Decision No 2455/2001/EC of 20 November 2001 establishing the list of priority substances in the field of water policy. *Off J Eur Commun* L 331, 15/12/2001, p 0001–0005.
10. O'Connor TP. 1994. The National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Mussel Watch Program: National monitoring of chemical contamination in the coastal United States. In Cothern CR, Ross NP, eds, *Environmental Statistics, Assessment and Forecasting.* Lewis, Boca Raton, FL, USA, pp 331–349.
11. Lauenstein GG, Cantillo AY. 1998. Sampling and analytical methods of the National Status and Trends program. Mussel Watch project: 1993–1996 update. Technical Memorandum NOS/ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD, USA.
12. Krahn MM, Wigren CA, Pearce RW, Moore LK, Bogar RG, MacLeod WD Jr, Chan SL, Brown DW. 1988. Standard analytical procedures of the NOAA National Analytical Facility, 1988: New HPLC cleanup and revised extraction procedures for organic contaminants. Technical Memorandum NMFS F/NWC 153. National Oceanic and Atmospheric Administration, Silver Spring, MD, USA.
13. Cantillo AY, Lauenstein GG. 1993. Performance based quality assurance of the NOAA National Status and Trends Projects. Intercomparison exercise results 1991–1993. In Parkany M, ed, *Quality Assurance for Analytical Laboratories.* The Royal Society of Chemistry, Cambridge, UK, pp 34–43.
14. Cantillo AY, Parris RM. 1993. Quality assurance project: Intercomparison exercise results 1991–1993. In Parkany M, ed, *Quality Assurance for Analytical Laboratories.* The Royal Society of Chemistry, Cambridge, UK, pp 44–52.

15. Cantillo AY. 1995. Standard and reference materials for environmental science (part 1 and part 2). Technical Memorandum NOS/ORCA 94. National Oceanic and Atmospheric Administration, Silver Spring, MD, USA.
16. Parris RM, Schantz MM, Wise SA. 1997. NIST/NOAA NS&T/EPA EMAP intercomparison exercises program for organic contaminants in the marine environment: Description and results of 1997 organic intercomparison exercises. Technical Memorandum NOS/ORCA 133. National Oceanic and Atmospheric Administration, Silver Spring, MD, USA.
17. Law RJ, Klungsoyr J. 1996. The 1994 QUASIMEME laboratory-performance studies: Polycyclic aromatic hydrocarbons (PAH) in standard solutions. *Mar Pollut Bull* 32:667–673.
18. Law RJ, Klungsoyr J, Freriks IL. 1997. The QUASIMEME laboratory performance study of polycyclic aromatic hydrocarbons (PAH): Assessment of the first three rounds, 1994–1995. *Mar Pollut Bull* 5:64–77.
19. Rao CR. 1964. The use and interpretation of principal component analysis in applied research. *Sankhya Ser A* 26:329–357.
20. Colombo JC, Pelletier E, Brochu C, Khalil M. 1989. Determination of hydrocarbon sources using *n*-alkane and polyaromatic hydrocarbon distribution indexes. Case study: Rio de la Plata estuary, Argentina. *Environ Sci Technol* 23:888–894.
21. Hites RA, Laflamme RE, Windsor JG. 1980. Polycyclic aromatic hydrocarbons in marine/aquatic sediments: Their ubiquity. *Adv Chem Ser* 185:289–311.
22. Bouloubassi I, Saliot A. 1993. Investigation of anthropogenic and natural organic inputs in estuarine sediments using hydrocarbon markers. *Oceanol Acta* 16:145–161.
23. Baumard P, Budzinski H, Garrigues P, Dizer H, Hansen PD. 1999. Polycyclic aromatic hydrocarbons in recent sediments and mussels (*Mytilus edulis*) from the Western Baltic Sea: Occurrence, bioavailability and seasonal variations. *Mar Environ Res* 47:17–47.
24. Meador JP, Stein JE, Reichert WL, Varanasi U. 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Rev Environ Contam Toxicol* 143:79–165.